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Response Surface Optimization of *Nigella glandulifera* Freyn Seed Oil Yield by Supercritical Carbon Dioxide Extraction

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Abstract

Supercritical carbon dioxide (SC-CO_2) extraction was employed to extract oil from *Nigella glandulifera* Freyn seed in this study. Response surface methodology (RSM) was applied to evaluate the effects of the process parameters (pressure, temperature, and CO_2 flow rate) on oil yield of *N. glandulifera* seed. A Box-Behnken design was used to optimize the extraction parameters. The analysis of variance indicated that the linear coefficients of pressure and CO_2 flow rate, the quadratic term coefficients of pressure and temperature and the interactions between pressure and temperature, as well as temperature and CO_2 flow rate, had significant effects on the oil yield ($P<0.05$). The optimal conditions to obtain the maximum oil yield from *N. glandulifera* seed were pressure 30.84 MPa, temperature 40.57°C, and CO_2 flow rate 22.00 L h^{-1}. Under these optimal conditions, the yield of oil was predicted to be 38.19%. The validation experiment results agreed with the predicted values. The fatty acid composition of *N. glandulifera* seed oil extracted using SC-CO_2 was compared with that of oil obtained by Soxhlet method. The results showed that the fatty acid compositions of oil extracted by the two methods were similar. Identification of oil compounds with gas chromatography-mass spectrometry (GC-MS) showed that the contents of unsaturated fatty acids linoleic acid (48.30%), oleic acid (22.28%) and saturated fatty acids palmitic acid (16.65%), stearic acid (4.17%) were the most abundant fatty acids in seed oil from *N. glandulifera*.

Key words: supercritical carbon dioxide extraction, *Nigella glandulifera* Freyn seed oil, response surface methodology, gas chromatography-mass spectrometry, fatty acids

INTRODUCTION

Nigella glandulifera Freyn, a plant of *Nigella* genus in Ranunculaceae family, is widely distributed in Xinjiang, Yunnan, and Tibet of China. The seeds of *N. glandulifera* are well-known as a Uighur's traditional medicine and food, which are included in Pharmacopoeia of the People's Republic of China from 1997 to now. These seeds are believed to have diuretic, analgesic, spasmolytic, galactagogue, and bronchodilator functions to cure edema, urinary calculus, and bronchial asthma (Xiao *et al*. 2002). They contain about 35-42% oil, which is rich in linoic acid and oleic acid. Linoic acid and oleic acid are unsaturated fatty acids, and are considered to be beneficial to the health of mankind, so its oil has high value for exploitation and utilization and can be used in foods, pharmaceuticals, and cosmetic formulations and so on.

Oil extraction using supercritical carbon dioxide (SC-CO_2) has gained increasing attention over the traditional techniques, like steam distillation and solvent extraction. SC-CO_2 has the advantages of using nontoxic, nonexplosive and volatile solvent, which protects extracts from

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thermal degradation and solvent contamination (Brunner 1994). There are reports on SC-CO_2 extraction as an excellent alternative to the use of chemical solvents in the extraction of oils from different plants such as corn (List *et al*. 1984), soybean (Friedrich *et al*. 1982) and cotton seeds (Kuk and Hron 1994; Bhattacharjee *et al*. 2007), cocoa beans (Saldaña *et al*. 2002), green tea (Chang *et al*. 2000), and ginseng (Wang *et al*. 2001). However, no studies have been reported on the oil extraction from *N. glandulifera* seed by SC-CO_2 extraction to our knowledge. Response surface methodology (RSM) which combines mathematics with statistics is often used to design experiments, build models, and evaluate the effects of factors (Yue *et al*. 2008). The main advantage of RSM is the small number of experimental trials needed to evaluate multiple parameters and their interactions (Chow *et al*. 1998), and it has been used successfully in food processing operations (Hierro and Santa-Maria 1992; Reverchon 1997; Lee *et al*. 2000; Huang *et al*. 2008; Liu *et al*. 2009). SC-CO_2 was used to extract seed oil from *N. glanelulifera* in this work. The effects of independent factors (pressure, temperature, and CO_2 flow rate) on the oil yield of *N. glandulifera* seed were investigated. RSM was employed to build a model between the oil yield and these independent factors as well as to develop a model equation that will predict and determine the optimum conditions for the oil yield.

RESULTS AND DISCUSSION

Model fitting

Oil yields obtained from all the experiments are listed in Table 1. The experimental data were used to calculate the coefficients of the second-order polynomial equation. The application of RSM offered, based on parameter estimates, an empirical relationship between the response variable, and the test variables under consideration. By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

$$Y=36.81+0.78x_1-0.014x_2+1.27x_3+0.41x_1x_2-0.099x_1x_3+0.32x_2x_3-2.16x_1^2-1.64x_2^2+0.031x_3^2 \quad (1)$$

The regression analysis of the response function with statistical analysis are given in Table 2. Statistical testing of the model was performed in the form of ANOVA. Here, the value for F and P (probability) ($P<0.05$ when significant) were 80.5708 and 0.0001, respectively, and the lack-of-fit of 0.7117 was not significant ($P>0.05$), indicating that the generated model adequately explained the data variation and significantly represented the actual relationship between the reaction parameters. The determination coefficient $R^2=0.9904$ indicating that 99.04% of the variability in the response could be explained by the model. From the P-values of each model term, we concluded that the linear coefficients of pressure and CO_2 flow rate and the quadratic terms of pressure and temperature, had highly significant effects on the oil yield at the 1% level ($P<0.01$). The interactions between pressure and temperature, as well as temperature and CO_2 flow rate, had significant effects on the oil yield at the 5% level ($P<0.05$).

Response surface analysis

From Eq. (1) we can see that the oil yield of *N. glandulifera* seed has a complex relationship with independent variables. The best way of expressing the effects of independent variables on the oil yield within the experimental space under investigation is to generate response surface plots of the equation. The three-dimensional response surfaces curves and corresponding contour plots were obtained using the Design Expert and are shown in Figs. 1, 2, and 3 to illustrate the relationship between independent variables and the oil yield.

Fig. 1 shows response surface curve and its contour plot for the effects of pressure and temperature on the oil yield and their interaction at a fixed flow rate of 20 L h^{-1}. The extraction pressure and temperature showed a quadratic effect on the response. At low pressure, the oil yield was increased with the increase of pressure. This is most likely due to the improvement of oil solubility resulted from the increased CO_2 density with the rise of pressure (Lee *et al*. 2000). When the pressure was increased to levels greater than approximately 30 MPa, the negative quadratic effect began to have an impact. Such effect of pressure is not unexpected, when the pressure becomes too high, a reduction in the solvent diffusivity and mass transfer

Table 1 Box-Behnken design and response for the oil yield of *N. glandulifera* seed

Trial no.	Factors			Responses	
	Pressure (x_1, MPa)	Temperature (x_2, °C)	CO_2 flow rate (x_3, L h^{-1})	Predicted (%)	Observed (%)
1	-1 (25)	0 (40)	1 (22)	35.270	35.280
2	-1 (25)	0 (40)	-1 (18)	32.358	32.540
3	-1 (25)	1 (45)	0 (20)	31.906	31.818
4	-1 (25)	-1 (35)	0 (20)	32.765	32.662
5	0 (30)	0 (40)	0 (20)	36.377	36.811
6	0 (30)	-1 (35)	-1 (18)	34.345	34.266
7	0 (30)	0 (40)	0 (20)	36.799	36.811
8	0 (30)	0 (40)	0 (20)	37.091	36.811
9	0 (30)	0 (40)	0 (20)	37.099	36.811
10	0 (30)	1 (45)	-1 (18)	33.690	33.597
11	0 (30)	0 (40)	0 (20)	36.687	36.811
12	0 (30)	-1 (35)	1 (22)	36.075	36.168
13	0 (30)	1 (45)	1 (22)	36.704	36.783
14	1 (35)	0 (40)	-1 (18)	34.299	34.290
15	1 (35)	-1 (35)	0 (20)	33.309	33.398
16	1 (35)	1 (45)	0 (20)	34.085	34.188
17	1 (35)	0 (40)	1 (22)	36.817	36.636

Table 2 Analysis of variance for the fitted quadratic polynomial model

Source	Sum of squares	*df*	Mean square	*F*-value	*P*-value
Model	51.5472	9	5.7275	80.5708	<0.0001
x_1	4.8221	1	4.8220	67.8341	<0.0001
x_2	0.0015	1	0.0015	0.0209	0.8891
x_3	12.9388	1	12.9388	182.0156	<0.0001
x_1x_2	0.6684	1	0.6683	9.4014	0.0182
x_1x_3	0.03881	1	0.0388	0.5460	0.4840
x_2x_3	0.4122	1	0.4122	5.7981	0.0469
x_1^2	19.5706	1	19.5706	275.3080	<0.0001
x_2^2	11.3029	1	11.3029	159.0027	<0.0001
x_3^2	0.0041	1	0.0041	0.0581	0.8164
Residual	0.4976	7	0.0711		
Lack of fit	0.1324	3	0.0441	0.4833	0.7117
Pure error	0.3652	4	0.0913		
Cor total	52.0448	16			
		R-squared: 0.9904		Adj R-squared: 0.9781	

coefficient will occur, which will offset the increase in extraction rate caused by higher CO_2 density (Clifford 1999; Xu *et al*. 2008; Liu *et al*. 2009). At low temperature, the oil yield was increased with the increase of temperature. This is most likely due to the increased mass transfer speed. However, at higher temperature levels (approximately 40°C), the oil yield was decreased with the further increase of temperature. This is most likely due to the reduced density of carbon dioxide, with a consequent reduction of solute solubility. For a volatile solute, there is competition between its solubility in SC-CO_2 and its volatility (Pourmortazavi and Hajimirsadeghi 2007). In this case, the optimum extraction pressure and temperature for the maximum yield of oil were around 30 MPa and 40°C.

The interaction between the extraction pressure and CO_2 flow rate is shown in Fig. 2 at the fixed extraction temperature of 40°C. How pressure affect the oil yield has been described in Fig. 1. The CO_2 flow rate showed a positive and significant effect on the oil yield of *N. glandulifera* seed. At a definite extraction pressure, the yield of oil was increased slightly with the increase of the CO_2 flow rate, and nearly reached a peak at the highest flow rate tested. It might be due to the decrease of resistance force in mass transfer with the increase of flow rate (Yin *et al*. 2005). So, the optimum flow rate for the maximum yield of oil was around 22 L h^{-1}.

Fig. 3 shows the effect of the extraction temperature and the CO_2 flow rate on the oil yield at a fixed extraction pressure of 30 MPa. How temperature and CO_2 flow rate affect the oil yield has been described in

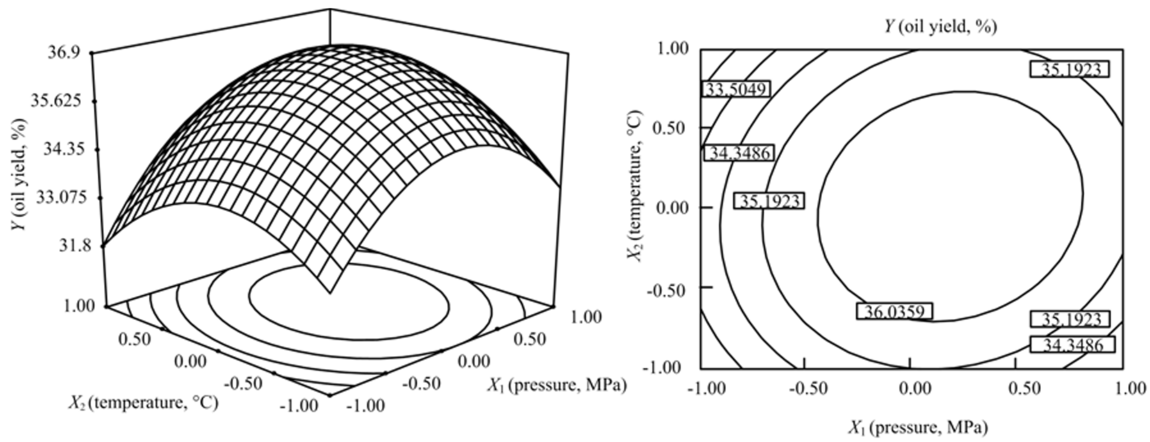

Fig. 1 Response surface curve and its contour plot for the effects of pressure and temperature at a constant CO_2 flow rate of 20 L h^{-1} on the oil yield.

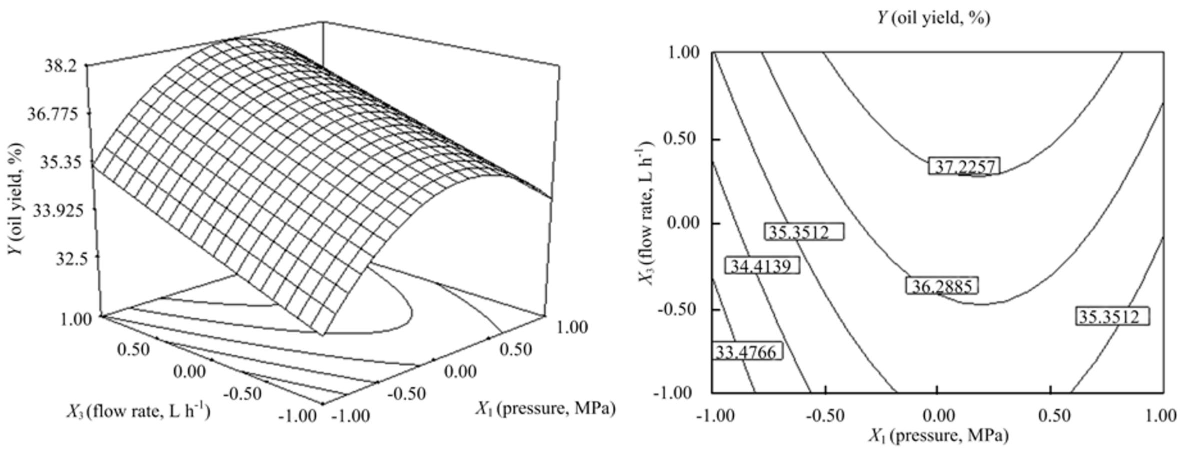

Fig. 2 Response surface curve and its contour plot for the effects of pressure and CO_2 flow rate at a constant extraction temperature of 40°C on the oil yield.

Figs. 1 and 2, respectively. At a definite extraction temperature, the CO_2 flow rate displayed a linear effect on the response in Fig. 3, there was a sharp increase of oil yield from about 33.7 to 36.0 % as the flow rate was increased from 18 to 22 L h^{-1}.

From these three-dimensional response surface curves and corresponding contour plots, it is evident that the CO_2 flow rate is the most significant factor affecting the oil yield in SC-CO_2 extraction, followed by the extraction pressure and temperature. The interactions of extraction pressure and temperature, as well as extraction temperature and CO_2 flow rate are significant for the oil extraction from *N. glandulifera* seed, compared to the interaction of extraction pressure and CO_2 flow rate.

By solving the inverse matrix with Software Design Expert, the optimum levels of the tested factors were extraction pressure at 30.84 MPa, extraction temperature at 40.57°C, and flow rate at 22 L h^{-1}. Under these conditions, the maximum predicted yield was 38.19%, and the observed yield was about 37.87-38.25%, the experimental values agreed with the predicted values.

Verification of the predictive model

In order to validate the model Eq. (1), a total of 15 verification experiments were carried out under different combinations of pressure, temperature, and time

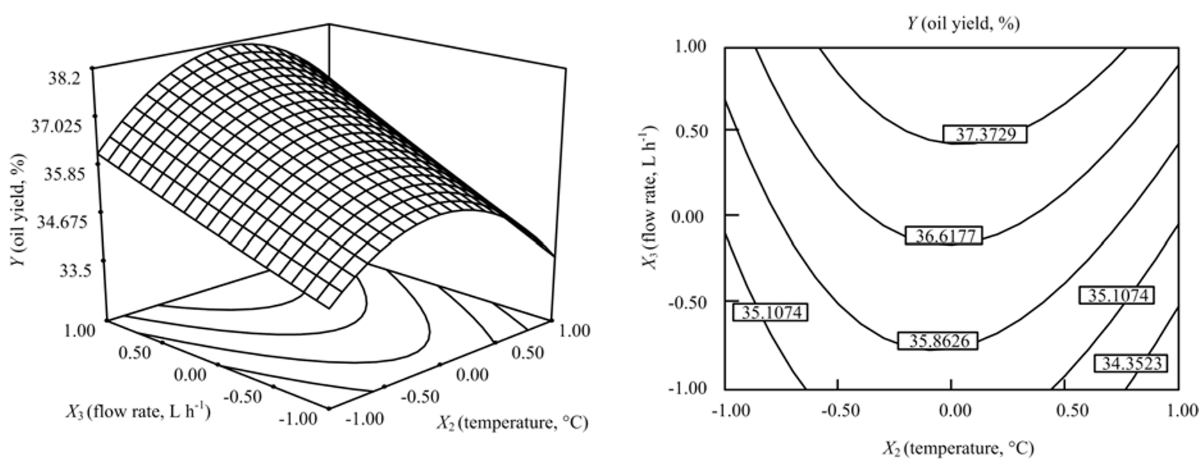

Fig. 3 Response surface curve and its contour plot for the effects of temperature and CO_2 flow rate at a constant extraction pressure of 30 MPa on the oil yield.

and the result is shown in Fig. 4. The plot demonstrates that the experimental points are evenly distributed around the diagonal of horizontal and vertical axis, which indicates that the experimental values are in good agreement with the predicted values, and also suggests that the predicted second order polynomial model is accurate and reliable. Thus, a statistically significant multiple regression relationship between the independent variables (pressure, temperature, and CO_2 flow rate) and the response variable (oil yield) was established. The second order polynomial model could therefore be effectively used to represent the relationship among the parameters selected.

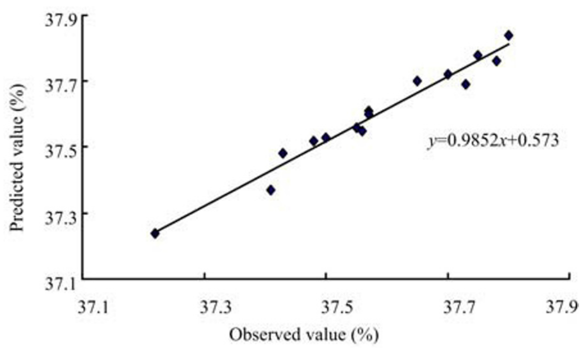

Fig. 4 Observed values vs. predicted values for model verification.

Fatty acid composition of *N. glandulifera* seed oil

The fatty acid composition of *N. glandulifera* seed oil extracted by SC-CO_2 and by Soxhlet method was determined by gas chromatography-mass spectrometry (GC-MS) and is shown in Table 3. The result shows that the fatty acid composition of oil extracted by the two methods is similar, and the content of linoleic acid and oleic acid reached up to 48.30 and 22.28%, respectively. As can be observed, the composition and content in fatty acids do not depend on the extraction method. The oil contained 12 fatty acids, among them, linoleic acid was the most abundant unsaturated fatty acids. This work also shows that *N. glandulifera* Freyn seed oil is a rich source of linoleic acid. Linoleic acid has the ability to inhibit platelet aggregation, which is consistent with the effect of *N. glandulifera* seed in activating blood circulation. The essential fatty acids like linoleic acid are not easily synthesized in the human body and must be supplied externally through the diet, and *N. glandulifera* seed oil can be a good nutritional supplement as a source of linoleic acid.

CONCLUSION

RSM was successfully applied for optimization of SC-CO_2 extraction parameters for *N. glandulifera* seed oil yield. The response surface plots indicated that the three factors (pressure, temperature and CO_2 flow rate) significantly influenced the oil yield, independently and interactively. The optimum process parameters were obtained as: pressure 30.84 MPa, temperature 40.57°C, and CO_2 flow rate 22 L h^{-1}. Under these conditions, the

Table 3 Fatty acid compositiona (%) of *N. glandulifera* seed oil extracted with SC-CO_2 extraction and Soxhlet extraction

Fatty acid composition (%)[1)]	SC-CO_2 extraction	Soxhlet extraction (8 h)
Myristic acid	0.18	0.18
9-Hexadecenoic acid	0.28	0.28
Heptadecanoic acid	0.24	0.12
Palmitic acid	16.65	16.71
Linoleic acid	48.30	47.85
Oleic acid	22.28	22.82
7-Octadecenoic acid	2.20	2.13
stearic acid	4.17	4.13
10,13-Octadecadienoic acid	0.39	0.37
Arachidic acid	0.15	0.18
11,13-Eicosadienoic acid	4.47	4.51
11-Eicosenoic acid	0.70	0.72

[1)] GC area percentage.

experimental values agreed with the predicted value. The adequacy of the predictive model was verified by the validation experiments. The fatty acids composition of *N. glandulifera* seed oil extracted by SC-CO_2 was similar to that of oil extracted by hexane.

MATERIALS AND METHODS

Materials

Seeds of *N. glandulifera* were purchased from Xinjiang Uigur Hospital at Urumqi in 2008, the moisture content of which was 6.6 wt% as determined by an infrared moisture meter (A-D4714, A&D Co. Ltd., Japan). The seeds were crushed to particle size of 0.45 mm. The carbon dioxide (99.5%) used in supercritical fluid extraction (SFE) was supplied by Hongjian Co. (Nanjing, China). All the chemicals used were of analytical grade.

Soxhlet extraction

Soxhlet extraction was carried out in triplicate for each experimental run, 10 g of *N. glandulifera* seed powder (with the particle size of 0.45 mm and moisture content of 6.6 wt%) was weighted and packed in a Soxhlet apparatus and then continuously extracted for 8 h at one time using n-hexane (60-80°C) as the solvent. After extraction, the solvent was evaporated by rotary vacuum evaporator (30°C) and the extract was dried at 103°C to remove residual solvent until a constant weight (Zaidul *et al*. 2006). The fatty acid components of the *N. glandulifera* seed oil obtained by Soxhlet extraction were analyzed and compared with the oil extracted by SC-CO_2.

Supercritical carbon dioxide extraction

Supercritical fluid extraction was performed on a Hua'an supercritical fluid extractor (Model HA121-50-01, Jiangsu, China). In each experiment, 150 g sample with particle size of 0.45 mm was loaded into a 1 000 mL extraction vessel. The operating conditions were set as follows: pressure (25-35 MPa), temperature (35-45°C), and CO_2 flow rate (18-22 L h^{-1}). The temperatures were controlled automatically, and the CO_2 flow rate and the pressures in both the extractor and separator were controlled manually using a back-pressure regulator. When the desired pressure, temperature, and CO_2 flow rate were reached, the extraction was started. In this experiment, we found that the oil yield reached to maximum within 30 min of extraction for all the experiments studied. This may be due to the fact that the extractable components were easily accessible by the solvent. So, each extraction run lasted for 60 min since longer extraction times did not significantly increase the yield of oil. The oil dissolved in the SC-CO_2 was discharged into the separator where carbon dioxide was depressurized; the oil was separated from the carbon dioxide and collected in the separator. The seed oil was recovered and the oil yield was determined gravimetrically.

Response surface experimental design

RSM was applied to optimize the operating conditions of SC-CO_2 extraction to obtain a high yield of oil from *N. glandulifera* seed in this study. Three independent variables studied were extraction pressure (X_1), extraction temperature (X_2) and CO_2 flow rate (X_3) for uncoded variable levels. These independent variables and their levels were selected based on our preliminary experiments (data not shown). Each independent variable was coded at three levels: -1, 0, and +1, their corresponding levels were as follows (low, medium, and high values): pressure of 25, 30, and 35 MPa; temperature of 35, 40, and 45°C; CO_2 flow rate of 18, 20, and 22 L h^{-1}. The dependent variable was the oil yield. The coded and corresponding uncoded independent variables used in the RSM design are listed in Table 4.

A Box-Behnken design was applied to optimize these three independent variables, and is shown in Table 1. In this study, the experimental design contained 17 trials and the value of the responses was the mean of triplications. Five replicates at the centre of the design were used for

Table 4 Independent variables and their levels for RSM design

Independent variable	Symbols		Level		
	Coded[1)]	Uncoded	-1	0	1
Pressure (MPa)	x_1	X_1	25	30	35
Temperature (°C)	x_2	X_2	35	40	45
CO_2 flow rate (L h^{-1})	x_3	X_3	18	20	22

[1)] $x_1=(X_1-30)/5$; $x_2=(X_2-45)/5$; $x_3=(X_3-20)/2$

estimation of a pure error sum of square. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors. A second-order polynomial model was used to express the oil yield (Y) as a function of the independent variables,

$$Y=B_0+B_1x_1+B_2x_2+B_3x_3+B_{12}x_1x_2+B_{13}x_1x_3+B_{23}x_2x_3+B_{11}x_1^2+B_{22}x_2^2+B_{33}x_3^2 \quad (2)$$

Where, Y is predicted response; B_0 is a constant; B_1, B_2, B_3 are linear coefficients; B_{12}, B_{13}, B_{23} are cross-product coefficients; and B_{11}, B_{22}, B_{33} are quadratic coefficients; x_1, x_2, x_3 are input variables.

A software Design-Expert 7.1.4 trial (Stat-Ease Inc., Minneapolis, MN, USA) was used to obtain the coefficients of the second-order polynomial model. The goodness of fit of the model was evaluated by the analysis of variance (ANOVA) and the coefficient of determination (R^2) (Chu *et al*. 2003).

Gas chromatography-mass spectrometry analysis

The preparation of fatty acid methyl ester (FAME) was carried out as follows: for saponification of free fatty acids, 5 g of the oil was added into 30 mL of 1 mol L^{-1} KOH/ethanol; after mixed well, 5 g of the mixture was dissolved in 30 mL of 2% (v/v) H_2SO_4/methanol for esterification of free fatty acids. The analysis of FAME was performed on an Agilent 5975 inert-GC-MSD equipped with a DB-5MS column (30 m×0.32 mm×0.25 μm). The sample (1 μL) was injected at a split ratio of 30:1 and the injector temperature was set at 280°C. The GC oven temperature was programmed isothermal at 60°C for 1 min, and then increased from 60°C to 280°C at 10°C min^{-1}. The final temperature was maintained for 50 min. The flame ionization detector (FID) was maintained at 230°C. The carrier gas was helium, at a flow rate of 0.8 mL min^{-1}. Electron impact mass spectra (70 eV) were acquired in the *m/z* range 20-550 amu. Then, the compounds were identified using mass spectrometric analysis. Spectra of the compounds were compared with those in the US National Institute of Standards and Technology Library (NIST02.L). The composition was expressed in percentage values calculated directly from GC peak areas, in a base without solvent and without applying correction factors. All above oil analysis was finished in the Modern Analysis Center of Nanjing University, China.

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